Kinetic aspects of the interaction of blood clotting enzymes

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VIII. The Relation between Clotting Time and Clotting Velocity

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Summary

It is shown that in a system where thrombin acts on fibrinogen, the clotting time can be used to assess coagulation velocity because clotting time and clotting velocity are inversely proportional.

Introduction

We have shown that the relation between coagulation time and prothrombin concentration in a one-stage prothrombin assay is

$$t_c = a.1/c + b \qquad (1)$$

where t_c = clotting time, c = prothrombin concentration and a, b = constants. The same type of relation very probably holds when c is the concentration of any of the coagulation factors V, VII, or X. (Hemker et al. 1967, 77). In a reaction catalyzed by an enzyme according to the Michaelis Menten model the reaction velocity is given by

$$1/v = a.1/c + b \qquad (2)$$

Comparison of (1) and (2) immediately suggests that clotting time might be inversely proportional to the velocity of coagulation. This article reports experiments to verify this suggestion. c may apply to either a substrate or an enzyme under the conditions prevailing in coagulation.

Theoretical Considerations

Reaction velocities are additive, that is: the result of two or more reaction velocities (v_1, v_2 etc.) yielding the same product is product formation (v_r), with a velocity equal to the sum of the constituent velocities. $v_r = v_1 + v_2$ etc., in general $v_r = \sum_{i=1}^{n} v_i$

When in the course of a reaction, the velocity is increased stepwise, then one can calculate the constant velocity which would have had the same effect as the discontinuous velocities that actually occurred. In Fig. 1 an initial velocity **c** is increased at moment **a** to the level **d**. Then at moment **b** the total effect of the two velocities is taken to be equivalent to one other

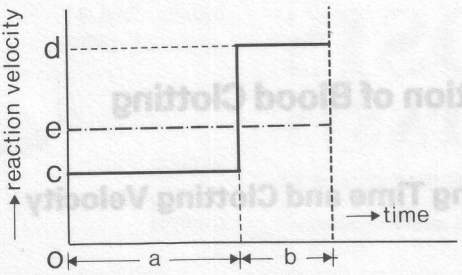

Fig. 1 Explanation in text.

velocity **e**. Because velocities are additive $e\,(a+b) = c \times a + d \times b$

from which it follows that

$$e = \frac{db + ca}{a + b} \tag{3}$$

In coagulation we can execute this experiment by adding a given amount of thrombin (T_o) at zero time, and after an interval (t_i) before clotting occurs, add a second amount to obtain a higher concentration of thrombin (T_n). Then one measures the clotting time t_{exp}. In an independent experiment one measures the clotting times that will be obtained by T_o or T_n added at zero time. We will call them t_o and t_n, respectively.

On the assumption that the clotting time is inversely proportional to clotting velocity we can translate this experiment in terms of Fig. 1, because (n is a proportionality constant) $t_o = n\ 1/c$; $t_n = n\ 1/d$ and $t_{exp} = a + b = n.1/e$. Applying this to formula (3) gives:

$$1/t_{exp} = \frac{1/t_n\,(t_{exp} - t_i) + 1/t_o.t_i}{t_{exp}}$$

from which it follows that

$$t_{exp} = t_n + t_i - (t_n/t_o)t_i$$

$$t_{exp} = t_n + \frac{(t_o - t_n)}{t_o}\,t_i \tag{4}$$

This formula can be put to test experimentally.

Fig. 2 Explanation in text.

Materials and Methods

The experiments were carried out in a thermostated (37°C) reaction vessel of a Radiometer pH Stat placed on a magnetic stirrer. As a stirring magnet a 5 mm steel rod was used. The vessel contained 500 µl Michaelis buffer pH 7.2 and 250 µl fibrinogen solution (Kabi fibrinogen 100 mg/ml). At zero time 50 µl of a thrombin solution were added (Thrombin Roche 50 U/ml or suitable dilution, kept at 0°C). At variable intervals before clotting occurred again 50 µl of thrombin were added. The moment of clotting was assessed by observing the first white thread that winds around the stirring magnet (Fig. 2).

In control experiments it could be shown that the dilution of the fibrinogen brought about by the second addition of thrombin did not change the coagulation time to a significant degree. The dilution brought about by the addition therefore was neglected.

Experimental

A representative experiment is shown in Table 1. In order to prevent systematic deviations each individual determination was assigned a random number and carried out in that order.

Table 1

Interval time (t_i) (sec)	0	5	10	15	20	25	30	35	∞
Coagulation times (t_{exp}) (sec)	26.8	28.5	31.2	33.3	35.4	38.5	40.3	42.2	49.7
	26.0	29.0	31.5	34.1	34.7	37.6	39.8	42.7	49.0
	27.0	28.7	31.3	33.5	35.2	37.5	39.6	42.5	49.1
	26.3	29.2	31.2	33.2	35.0	37.6	39.6	42.3	48.4
	26.6	28.3	31.4	32.6	35.4	37.7	40.1	42.0	49.8
	26.1	28.6	31.3	33.0	34.8	37.8	40.1	42.2	48.8
	26.3	28.8	31.9	33.3	35.9	37.5	40.0	41.9	48.9
	26.4	28.0	31.4	32.7	36.5	37.5	39.7	42.0	48.9
	27.3	28.6	30.5	33.5	36.0	38.5	39.9	41.7	49.4
	26.5	28.6	30.7	34.0	35.8	37.6	40.1	42.6	49.0
Mean	26.5	28.6	31.2	33.3	35.5	37.8	39.9	42.2	49.1
Standard deviation	0.4	0.3	0.4	0.5	0.6	0.4	0.3	0.3	0.4

At zero time 50 µl of a thrombin solution were added; after the interval time indicated the same amount was again added. Infinite interval times indicate no second addition. In terms of formula (4) we found $t_n = 26.5$ and $t_o = 49.1$. When t_{exp} is plotted as a function of interval time, Fig. 3 is obtained. We see that t_{exp} is a linear function of t_1. We measure a slope of 0.46. According to formula (4) this slope should be

$$\frac{t_o - t_n}{t_o} = \frac{49.1 - 26.5}{49.1} = 0.46.$$

In repeated experiments of the same type the slopes calculated from equation (4) were found to be equal within the experimental error.

Discussion

The evidence obtained does not counteract the derivations that are based on the assumption that clotting time is inversely proportional to clotting velocity. Any other type of

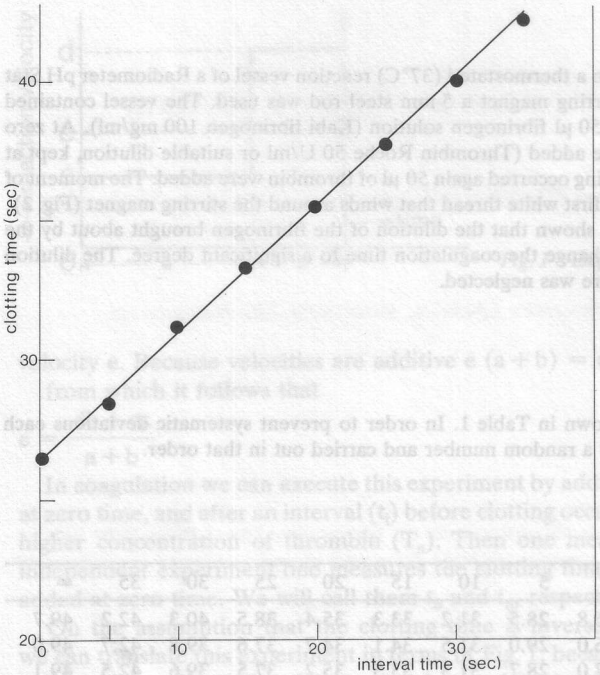

Fig. 3 Moment of clotting as observed in the reaction vessel.

relation between clotting time and clotting velocity would have led to different formula. We therefore cannot falsify the original assumption. Still it may be possible that a type of relationship which resembles $v = n.1/t_c$ very much (for instance $v = n.1/t_c + a$ with small a etc.) would describe the situation better. As the experimental limits of our method are the same as in those where the relationship will be used in practice, i.e. the measuring of clotting times, this will be of little practical importance.

Résumé

Il est démontré que dans un système où la thrombine agit sur le fibrinogène, le temps de coagulation peut être utilisé pour établir la vitesse de coagulation car le temps de coagulation et la vitesse de coagulation sont inversément proportionnels.

Zusammenfassung

Es wird gezeigt, daß in einem System, in dem Thrombin Fibrinogen umsetzt, die Gerinnungszeit zur Bestimmung der Koagulationsgeschwindigkeit herangezogen werden kann, da die Gerinnungszeit und die Gerinnungsgeschwindigkeit invers proportional sind.

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